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Effects of orally-bioavailable short-acting kappa opioid receptor-selective antagonist LY2456302 on nicotine withdrawal in mice

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ABSTRACT

Kappa opioid receptor (KOR) signaling has been implicated in mediating behavioral and biochemical effects associated with drug dependence. The most commonly used KOR antagonists, norbinaltorphimine (norBNI) and (3R)-7-Hydroxy-N{(1S)-1-{[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl] methyl}-2-methylpropyl}-1,2,3,4-tetrahydro-3-isoquinoline-carboxamide (JDTic), have provided a wealth of information in this area; however, the delayed onset and long-lasting effects of these antagonists complicate experimental design and interpretation of results, and make them less than ideal for clinical studies. Initial studies with the recently developed KOR antagonist, LY2456302, show that the compound is a short acting, high-affinity, selective KOR antagonist with therapeutic potential for mood disorders and ethanol use in animal models, and is well tolerated in humans. The goal of the current study was to evaluate the effectiveness of LY2456302 in alleviating the nicotine withdrawal syndrome in mice. Mice were chronically treated with nicotine for 14 days and physical and affective nicotine withdrawal signs were measured using a spontaneous nicotine withdrawal model and conditioned place aversion (CPA) following pre-treatment with LY2456302, administered orally. Vehicle treated nicotine withdrawn mice displayed significant anxiety-related behavior, somatic signs, hyperalgesia, and CPA. Similar to previous studies with norBNI and JDTic, LY2456302 alleviated the nicotine withdrawal syndrome, as evidenced by decreased expression of nicotine withdrawal induced anxiety-related behavior, somatic signs, and CPA, and increased hotplate latency in nicotine withdrawn mice following pre-treatment. Given the current results, and with its favorable pharmacokinetic and pharmacodynamic profile, LY2456302 may be a useful therapeutic agent for treatment of multiple aspects of the nicotine withdrawal syndrome.

1. Introduction

Scientific evidence increasingly supports a role for kappa opioid receptor (KOR) signaling in mediating the behavioral and biochemical effects associated with aversive and depressive-like states, and substance use dependence. The mechanism of KOR involvement in regulating motivational and emotional states has been suggested to involve dynorphin, the endogenous KOR ligand. Rewarding and stressful stimuli increase cyclic adenosine monophosphate response element binding protein (CREB), resulting in increased levels of dynorphin, which have been observed after stress or drug exposure (Nestler et al., 2002). The increased levels of dynorphin bind to the KOR, resulting in reduced levels of dopamine and a state of anhedonia. Blockade of KOR by antagonists alleviates negative motivational and emotional states through blocking dynorphin interactions with the receptor (Carroll and Carlezon, Jr., 2013). In particular, disruption of KOR function attenuates stress responses, which can serve as an environmental trigger for neuropsychiatric conditions, such as depressive disorders and addiction (Van't Veer and Carlezon, Jr., 2013). The prototypical KOR antagonists, norbinaltorphimine (norBNI) and (3R)-7-Hydroxy-N{(1S)-1-{[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl] methyl}-2-methylpropyl}-1,2,3,4-tetrahydro-3-isoquinoline-carboxamide (JDTic), and the selective KOR agonist, U50,488, have been used to assess KOR involvement in these effects, and have provided much of the current knowledge in this area.

In rodent models of drug withdrawal, norBNI and/or JDTic attenuated nicotine and morphine somatic signs of withdrawal

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(Tejeda et al., 2012; Jackson et al., 2010; Kelsey et al., 2015), ethanol and nicotine withdrawal-induced anxiety-related behavior as measured by the elevated plus maze (Schank et al., 2012; Valdez and Harshberger, 2012; Gillett et al., 2013; Jackson et al., 2010), nicotine and morphine withdrawal conditioned place aversion (CPA) (Jackson et al., 2010; Kelsey et al., 2015), and ultrasonic vocalizations associated with ethanol withdrawal (Berger et al., 2013). JDTic and norBNI also decreased ethanol self-administration in rats (Schank et al., 2012; Walker and Koob, 2008; Walker et al., 2011). Many of these behaviors were exacerbated by treatment with U50,488 (Valdez and Harshberger, 2012; Berger et al., 2013; Schank et al., 2012; Gillett et al., 2013; Tejeda et al., 2012). Alternatively, administration of the peripheral KOR agonist, ICI 204,448, inhibited nicotine withdrawal induced increases in feeding, metabolism, and locomotor activity in rats (Sudakov et al., 2014), suggesting that peripheral and central KOR signaling may differentially mediate nicotine withdrawal.

Despite their utility in understanding KOR involvement in addictive behaviors, various confounds affecting experimental design and interpretation of results are evident with these antagonists. First, the onset of KOR antagonism by norBNI and JDTic can be delayed for hours (Munro et al., 2012; Carroll et al., 2004). Both compounds also have very long durations of action, which can result in an extended pharmacodynamic effects or increase the potential for undesirable drug–drug interactions. NorBNI and JDTic have been shown to block antinociceptive activity in mice for up to 2 weeks (Carroll et al., 2004; Patkar et al., 2013), and significant KOR antagonist activity is detectable in rodents for up to 28 days (Munro et al., 2012; Carroll et al., 2004; Patkar et al., 2013). Such drug properties would also be unfavorable for clinical use. LY2456302, a recently developed, potent, high-affinity selective KOR antagonist, which has a plasma half −life of 2–4 h in rats and mice and receptor occupancy <50% within 48 h after oral administration (Rorick-Kehn et al., 2014), may be a more useful tool for assessing the therapeutic potential of KOR modulation as treatment for addictive disorders in preclinical and clinical models.

Although literature is limited, LY2456302 has shown promise in initial preclinical and clinical studies. LY2456302 reversed KOR agonist-induced analgesia and prepulse inhibition in rats, reduced immobility in the mouse forced swim test, and reduced ethanol self-administration in rats, with no significant effects on motor performance, thermoregulation, or dopamine levels in the nucleus accumbens (Rorick-Kehn et al., 2014). In a clinical study, acute and chronic (14 day) administration of LY2456302 was generally well tolerated in healthy participants, and had a favorable pharmacokinetic profile, supporting its development for therapeutic use in psychiatric disorders (e.g., depression, anxiety, substance use) (Lowe et al., 2014). LY2456302 has recently advanced to phase II clinical trials for augmentation of antidepressant therapy in treatment-resistant depression and is currently being clinically evaluated for use in mood and anxiety spectrum disorders (ClinicalTrials.gov, 2014). Because of its ability to reduce ethanol self-administration, similar to norBNI and JDTic, LY2456302 may also have therapeutic effects on other drug-induced behaviors.

The goal of this study was to evaluate the effectiveness of the novel KOR antagonist, LY2456302, in established models of nicotine withdrawal in mice, and to determine if a short acting KOR antagonist would show the same effects in a nicotine withdrawal study as previously tested longer acting KOR antagonists, JDTic and norBNI (Jackson et al., 2010). Interest in LY2456302 as a potential therapeutic agent in the treatment of nicotine withdrawal is also heightened since the drug is the only KOR to proceed through to a phase II clinical study. Mice were chronically treated with nicotine for two weeks, and spontaneous nicotine withdrawal behaviors were measured 18–24 h after mini pump removal, and 60 min after pretreatment with orally-administered LY2456302. The ability of LY2456302 to attenuate expression of nicotine withdrawal CPA was also tested.

2. Materials and methods

2.1. Animals

Naïve male 8-10 week-old ICR mice (Harlan Laboratories; Indianapolis, IN) served as subjects. Mice were housed five per cage in a 21 °C humidity controlled facility with ad libitum access to food and water. The animal facility was approved by the Association for Assessment and Accreditation of Laboratory Animal Care. Experiments were performed during the light cycle and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. Drugs

(−)-Nicotine hydrogen tartrate salt [(−)-1-methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate salt] was purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). (S)-3-fluoro-4-(4-((2-(3,5-dimethylphenyl)pyrrolidin-1-yl)methyl)phenoxy) benzamide (LY2456302) was supplied by the National Institute on Drug Abuse (Washington, DC, USA). The doses of LY2456302 used in our study are within the range of doses used to assess in vivo KOR effects as reported in the literature (Rorick-Kehn et al., 2014). Nicotine was dissolved in physiological saline (0.9% sodium chloride). The pH of the nicotine solution was checked and neutralized if necessary. LY2456302, was dissolved in water with the addition of 2% methylcellulose. LY2456302 was given to mice orally via gavage at the volume of 10 ml/kg. Drugs were mixed fresh on the day of dosing. All doses are expressed as the free base of the drug.

2.3. Nicotine chronic administration protocol

Mice were anesthetized with sodium pentobarbital (45 mg/kg, intraperotineal) and implanted subcutaneoulsy (s.c.) with Alzet osmotic mini pumps [(model 2002); Durect Corporation, Cupertino, CA]. Mice received one dose of ketoprofen after the mini pump operation. The concentration of nicotine was adjusted according to animal weight and the mini pump flow rate to deliver 24 mg/kg/day for 14 days. The spontaneous nicotine withdrawal model for induction of nicotine dependence has generally used the 24 mg/kg/day nicotine dose, as this dose was shown to produce a significant withdrawal syndrome in a spontaneous model for all three behavioral tests when compared to a higher dose of 48 mg/kg/day (Damaj et al., 2003).

2.4. Spontaneous nicotine withdrawal

To assess the effects of acute LY2456302 administration on spontaneous withdrawal from nicotine, mice were infused with nicotine or saline for 14 days. On day 14, mice were anesthetized under isoflurane anesthesia and minipumps were removed in the evening. No analgesic was given after mini pump removal, as this would interfere with hyperalgesia testing.

Mice were habituated to the gavage procedure for oral drug administration for 3 days prior to mini pump removal. On day 15, 18–24 h after mini pump removal, these mice were pretreated orally (p.o.) 60 min prior to testing with either vehicle or LY2456302 (1, 3, and 10 mg/kg, p.o.). Immediately following the 60 min pretreatment, mice were observed for physical and affective nicotine withdrawal signs as described in Damaj et al. (2003). Mice were first evaluated for 5 min in the plus maze test for anxiety-related behavior. Time spent on the open arms of the plus maze was assessed as a measure of anxiety-related response. The number of arm crosses between the open and closed arms was also counted as a measure of locomotor activity. The plus maze assessment was immediately followed by a 20-min observation of somatic signs measured as paw and body tremors, head shakes, backing, jumps, curls, and ptosis. Mice were placed in clear activity cages without bedding for the observation period. The total number of somatic signs was tallied for each mouse and the average number of somatic signs during the observation period was plotted for each test group. Hyperalgesia was evaluated using the hot plate test immediately following the somatic sign observation period. Mice were placed into a 10-cm wide glass cylinder on a hot plate (Thermojust Apparatus, Richmond, VA) maintained at 52 °C. The latency to reaction time (jumping or paw licking) was recorded. The specific testing sequence was chosen based on our prior studies showing that this order of testing reduced within-group variability and produced the most consistent results (Jackson et al., 2008). All studies were performed by an observer blinded to experimental treatment.

2.5. Nicotine withdrawal in the CPA test

The CPA protocol was conducted over the course of four days in a biased fashion as described in Jackson et al. (2009). Briefly, mice were implanted with 28-day nicotine (36 mg/kg/day) or saline mini pumps 14 days prior to initiation of CPA testing to induce dependence. Infusion continued throughout the duration of testing. The nicotine withdrawal CPA model was developed using the dose of